

# SELF-RADIATION PRODUCTS FORMED FROM 3,5,3'-TRI-iodo-L-THYRONINE



3,5,3'-tri-iodo-L-tyronine, labelled with  $^{131}\text{I}$  is used in our laboratories mainly for two purposes, namely, for metabolic studies and for red-cell uptake as an additional indicator of thyroid activity.<sup>2</sup> Because of its marked influence on metabolism, low concentrations of the thyroid hormone are required for metabolic studies. This necessitates the preparation of thyroid hormones of extremely high specific radioactivity. The test for red-cell uptake depends upon the affinity of red cells for thyroid hormones. This affinity is less than that of the serum proteins. As thyroid activity increases, increased concentration of circulating thyroid hormone causes saturation of the binding protein in serum, and as a result more and more

hormone is taken up by the red cells. Since red-cell uptake therefore clearly depends on the concentration of the hormone, a small concentration of the hormone with high radioactivity is again required.

In South Africa we still depend largely on the import of labelled hormones of high specific radioactivity. These hormones reach our laboratories, at the earliest, 5 days after production (day of production is taken as the day of determination of specific activity) and sometimes only a few days before the expiry date (14 days after production). During transport and storage, radiochemical decomposition from self-radiation<sup>3</sup> may occur. It is conceivable that the extent of decomposition bears a relationship to the specific radioactivity.

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The possible processes leading to the formation of artefacts are as follows:

(a) De-iodination which could result in the formation of  $^{131}\text{I}$ , 3,5-di-iodothyronine and, less probably, to 3-mono-iodothyronine.

(b) The oxidation of  $^{131}\text{I}$  to  $^{131}\text{I}_2$  which could re-iodinate the de-iodinated thyronines to labelled tri-iodothyronine and thyroxine.

(c) The introduction of chemical changes in the alanine side-chain which would result in the formation of the lactic acid analogues.<sup>3</sup>

In this study, 3,5,3'-tri-iodo-L-thyronine, labelled with  $^{131}\text{I}$ , was analyzed quantitatively for the formation of decomposition products at fortnightly intervals up to 75 days by chromatographic, electrophoretic, and radio-autographic means. The hormone was obtained from Abbott Laboratories, Oak Ridge, Tennessee, USA, labelled by a method of radio-isotopic exchange<sup>1</sup> with  $^{131}\text{I}$  in the 3'-position only. The specific radioactivity of the samples used varied from 21.4 to 29.6 mc/mg., and the concentrations from 13.0 to 19.5  $\mu\text{g}$ . of tri-iodothyronine/ml. in 50% propylene glycol.

The 4 major radiochemical breakdown products formed were  $^{131}\text{I}$ ,  $^{131}\text{I}$ -labelled 3,5-di-iodothyronine,  $^{131}\text{I}$ -labelled thyroxine, and an hydroxy-acid analogue of tri-iodothyronine or of di-iodothyronine, or both. It was established that the chromatographic solvents used in this study were not responsible for these breakdown products. Quantitative analyses indicate that the

mechanism of radiochemical decomposition of  $^{131}\text{I}$ -labelled tri-iodothyronine proceeds as follows:

(i) De-iodination of the most labile position (3'-position).

(ii)  $^{131}\text{I}$  exchange at positions of 3 and/or 5 which renders the 3,5-di-iodothyronine radioactive.

(iii) Oxidation of  $^{131}\text{I}$  to  $^{131}\text{I}_2$ .

(iv) Iodination of  $^{131}\text{I}$ -labelled tri-iodothyronine to labelled thyroxine.

(v) Oxidative de-amination followed by reduction of the side chain.

It is concluded that the danger exists of interpreting a self-induced radiation product as a metabolic transformation. Furthermore, if a stored sample of  $^{131}\text{I}$ -labelled tri-iodothyronine is used for studies of red-cell uptake, a correction factor is required.

In our laboratory we now prepare our own  $^{131}\text{I}$ -labelled thyroid hormones. The main difficulty is encountered with the specific radioactivity determination in samples in which the chromatographically pure product is too small to be weighed accurately. To overcome this problem we determine the concentration of purified thyroid hormone by stable iodine ( $\text{I}^{127}$ ) analyses, and the radioactivity ( $^{131}\text{I}$ ) by counting.

#### REFERENCES

1. Gleason, G. I. (1955); *J. Biol. Chem.*, **213**, 837.
2. Hamolsky, M. W., Stein, M. and Freedberg, A. S. (1957); *J. Clin. Endocrinol.*, **17**, 33.
3. Tata, J. (1959); *Clin. Chim. Acta*, **4**, 427.